JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1995, p. 2102–2106 Vol. 33, No. 8
0095-1137/95/$04.00+0

Distribution of IS*901* in Strains of *Mycobacterium avium* Complex from Swine by Using IS*901*-Detecting Primers That Discriminate between *M. avium* and *Mycobacterium intracellulare*

KEI NISHIMORI,[1]* MASASHI EGUCHI,[1] YUJI NAKAOKA,[2] YUKA ONODERA,[3] TAKASHI ITO,[1] AND KIYOSHI TANAKA[1]

Hokkaido Branch, National Institute of Animal Health, Hitsujigaoka-4, Toyohira, Sapporo, 062,[1] Tokachi Livestock Hygiene Service Center, Kisen 59-6, Kawanishi, Obihiro, 089-11,[2] and Kamikawa Livestock Hygiene Service Center, 4-sen 15, Higashitakasu, Asahikawa, 071-13,[3] Japan

Received 12 August 1994/Accepted 4 May 1995

The presence of the mycobacterial insertion sequence IS*901* was studied by PCR with reference strains of *Mycobacterium avium* complex; 122 veterinary strains of mycobacteria, mainly *M. avium* complex, isolated from swine; and 15 clinical strains. Four kinds of DNA extraction methods for PCR were compared. Use of the commerical extraction matrix allowed for the faster and easier preparation of PCR-amplifiable DNA than use of NaOH heating extraction or sodium dodecyl sulfate extraction of pretreated mycobacteria. It also provided more effective protection than boiling extraction against the destruction of DNA. Four reference strains of serovars 1 to 3 possessed IS*901*. Nine reference strains of serovars 1, 4 to 6, 8 to 11, and 21 possessed only IS*901* insertion sites. A novel PCR product was found in the other reference strains of serovars 7, 12 to 17, 19, and 20 and two clinical strains of serovar 15. It is suggested that the primers that amplified the insertion portion of IS*901* divided the *M. avium* complex into *M. avium*, *Mycobacterium intracellulare*, and other mycobacteria. None of the 110 strains of *M. avium* complex isolated from swine possessed IS*901*. It is suggested that the absence of IS*901* might be characteristic of swine-derived strains of *M. avium* complex.

Mycobacterium avium complex (MAC) or *M. avium-Mycobacterium intracellulare* complex is a clinically and hygienically important atypical mycobacterium that causes tuberculosis-like diseases in humans, especially immunocompromised individuals including AIDS patients, and mycobacteriosis in animals, especially local granulomatous infections in swine and cattle (7, 10, 14). The sources of MAC infection in humans and domestic animals are unknown, but its presence in human, animal, bird, and environmental samples suggests a number of possible reservoirs (5).

MAC was initially classified into seroagglutination types: serovars 1 to 3 were designated *M. avium* and serovars 4 to 21 were designated *M. intracellulare*. The closely related phenotypes of these two species led to their being grouped into a single complex, MAC (20). Anz and collaborators (1) showed biologically that serovar 7 is *M. intracellulare* and that serovars 4 to 6 and 8 to 11 are an intermediate group between *M. avium* and *M. intracellulare*. Saito and collaborators (11), using the Gen-Probe Rapid Diagnosis System for MAC, showed that strains of serovars 1 to 6, 8 to 11, and 21 hybridized with the *M. avium* probe and that strains of serovars 7 and 12 to 20 hybridized with the *M. intracellulare* probe. These results were also supported by Baess (2) and Böddinghaus et al. (3) by DNA-DNA hybridization and sequence analysis of 16S rRNA, respectively. Recently, in a cooperative study (17), the International Working Group on Mycobacterial Taxonomy reached similar results when agglutination serovar designations, molecular criteria, and cultural properties were used. These MAC isolates with higher serovar numbers are most commonly associated with tuberculosis in patients with AIDS and with the recent swine tuberculosis outbreak, whereas the classical *M. avium* isolates (serovars 1 to 3) are those commonly associated with infections in birds and other animals (17).

Three subspecies of *M. avium* were recently classified (13) and distinguished by the DNA sequences of repetitive elements, designated IS*900*, IS*901*, and IS*902* (4, 6, 8). The specific system of detecting IS*901* by PCR was developed by Kunze et al. (5). Their analysis of MAC strains isolated from clinical, veterinary, and environmental sources demonstrated that IS*901* is specifically related to *M. avium* strains pathogenic for animals and, in contrast, that most clinical *M. avium* strains and all strains derived from patients with AIDS lacked IS*901* (5, 14).

Apart from a few strains from swine tested by Kunze et al. (5), the distribution of IS*901* in MAC isolates from swine has not been investigated. In the study described here, an easy method for the preparation of template DNA for PCR and the precise distributions of IS*901* in the reference strains and swine-derived strains were examined.

MATERIALS AND METHODS

Bacterial strains. The 35 reference strains of *M. avium-M. intracellulare-Mycobacterium scrofulaceum* and laboratory strains (see Table 2) were obtained and maintained as described previously (10). They were grown on slants of Ogawa medium or plates of Middlebrook 7H11 agar with oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, Mich.) (10). *M. avium* subsp. *paratuberculosis* (*M. paratuberculosis*) ATCC 19698 was grown on slants of Herrolds egg yolk medium with 2 μg of mycobactin J (Allied Monitor, Inc. Fayette, Mo.) per ml.

One hundred thirty-seven strains were examined. These strains were isolated from 1984 to 1993. Fifty-eight strains were isolated from swine with tuberculosis lesions or from environmental sources. These strains were obtained from six swine herds (in Kagosima, Hyogo, Ishikawa, Saitama, Yamagata, and Hokkai prefectures in Japan), and strain identification was accomplished by thin-layer chromatography and seroagglutination (10, 15, 21). Thirty-two strains were obtained from M. Okabe, Kensei Meat Inspection Center, Shimodate, Ibaraki, Japan, and 30 strains were obtained from M. Katsumi, Sendai Meat Inspection Center, Sendai, Miyagi, Japan. One strain from pigeons and one strain from

* Corresponding author. Mailing address: Hokkaido Branch, National Institute of Animal Health, Hitsujigaoka-4, Toyohira, Sapporo, 062, Japan. Phone: 11-851-2160. Fax: 11-853-0767. Electronic mail address: GBH01652@niftyserve.or.jp.

young cows were also examined. Fifty strains from humans were obtained from A. Sato, Environment and Hygiene Research Center of Kobe City, Kobe, Japan. The strains were stored at −80°C and were passaged once or twice on 7H11 agar plates to check their purity before use.

Preparation of mycobacterial DNA. (i) Method 1. DNA was extracted from enzymatically lysed organisms cultured in Middlebrook 7H9 medium, as described by Whipple et al. (19). *Mycobacterium* cultures were grown in 75-ml tissue culture flasks (Sumitomo Bakelite Co., Ltd, Tokyo, Japan) containing 75 ml of Middlebrook 7H9 liquid medium (pH 5.9) with OADC enrichment, 0.05% Tween 80, and ferric mycobactin J. Static cultures were incubated at 37°C until the turbidity (A_{540}) of each suspension was approximately 0.2. After sterilized D-cycloserine (Sigma Chemical Co., St. Louis, Mo.) was mixed in each flask to a final concentration of 1 mg/ml, all cultures were incubated for an additional period (*M. paratuberculosis*, 24 h; all others, 4 h). The cells were then harvested by centrifugation (11,000 × *g* for 30 min at 10°C) and were washed twice in 50 mM Tris hydrochloride (pH 8.0)–100 mM EDTA–150 mM NaCl (TEN).

Cells were sequentially treated with 16,000 U of lipase (L-4385; Sigma) for 2 h at 37°C, 5 mg of lysozyme (L-5876; Sigma) per ml for 2 h at 37°C, and 2 mg of proteinase K (P-0390; Sigma) per ml and 1% sodium dodecyl sulfate (SDS) for 16 h at 50°C as described by Whipple et al. (19). After a 0.4 volume of cold 5 M potassium acetate solution was gently mixed, samples were placed on ice for 10 min and centrifuged (16,000 × *g* for 10 min at 4°C). DNA was extracted and purified by repeated extraction with TEN-saturated phenol-chloroform-isoamyl alcohol (25:24:1; vol/vol/vol), extraction with chloroform-isoamyl alcohol (24:1; vol/vol), and precipitation of absolute ethanol, as described by Whipple et al. (19). The resultant DNA pellets were suspended in 50 to 60 μl of 10 mM Tris-hydrochloride–1 mM EDTA (pH 8.0) (TE). The final DNA concentration was determined by measuring the A_{258}.

(ii) Method 2. Mycobacterial colonies on agar media were suspended in 200 μl of 0.2 N NaOH (in 1.5-ml screw-cap Eppendorf tubes), and the cells were lysed at 120°C for 10 min as described by Vary et al. (16). DNA was extracted and purified as described above.

(iii) Method 3. DNA was extracted by using the InstaGene Purification Matrix (Bio-Rad Laboratories, Hercules, Calif.). Mycobacterial colonies were suspended in 200 μl of the matrix, and the mixture was incubated at 56°C for 30 min. The suspensions were vortexed at high speed for 10 s and were then placed in a boiling water bath for 8 min. Then, they were vortexed again and centrifuged at 17,000 × *g* for 3 min. The resultant supernatants were used for PCR. When not in use, the suspensions were stored at −20°C and were centrifuged again before use.

(iv) Method 4. The colonies were suspended in 200 μl of TE and were then boiled for 20 min in 1.5-ml screw-cap Eppendorf tubes to release the microbial DNA, as described by Moss et al. (9). The tubes were centrifuged at 17,000 × *g* for 5 min (9), and the supernatants were stored at −20°C until they were needed.

PCR primers and conditions. PCR was performed as described by Kunze et al. (5) with a GeneAmp PCR Reagent Kit with AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Briefly, the PCR conditions for IS*900*, IS*901*, and the insertion portion of IS*901* consisted of a 36-cycle reaction that included denaturation (94°C, 60 s), annealing (68°C, 60 s), and extension (72°C, 60 s) (5). A part of the template DNA sample in 25 μl of the PCR reaction volume was 2.5 μl, but 10 μl was used for methods 3 and 4.

Three kinds of primers were used. The primer pairs specific for *M. avium* restriction fragment length polymorphism (RFLP) type A/I that were derived from the sequence of IS*901* (primers A) and those specific for *M. avium* RFLP type A and *M. paratuberculosis* that were derived 150 bp upstream and downstream of the IS*901* insertion in *M. avium* RFLP type A/I (primers B) were described by Kunze et al. (5). In the study of Kunze et al. (5) the expected size of the former PCR product was 1,108 bp. The expected size of the latter PCR products was 300 bp for *M. avium* RFLP type A and *M. paratuberculosis* and 1,776 bp for *M. avium* RFLP type A/I. The primer pairs specific for *M. paratuberculosis* that were derived from IS*900* (primers C) were also used, for which the expected PCR product was 229 bp (16).

Agarose gel electrophoresis. The PCR products and DNA fragments digested by restriction endonuclease were separated by agarose gel electrophoresis at 4 V/cm for 60 to 75 min with minigel electrophoresis system (Mupid-2; Advance Co. Ltd., Tokyo, Japan) or a Computer Controlled Electrophoresis Power Supply (model 3000Xi; Bio-Rad Laboratories). The gel contained 2% agarose (LO3; TAKARA Shuzo Co., Ltd., Tokyo, Japan) in 89 mM Tris–89 mM borate–2 mM EDTA buffer or 80 mM Tris–phosphate–2 mM EDTA buffer. The gels were stained for 30 min in ethidium bromide (0.15 μg/ml in the electrophoresis buffer described above), rinsed in distilled water, and photographed under short-wave UV light. The DNA molecular weight standard marker, pHY Marker, was obtained from TAKARA Shuzo Co., Ltd.

Restriction endonuclease digestion. Two microliters of the PCR product was digested with individual restriction endonucleases in a 10-μl reaction mixture under the conditions specified by the manufacturer. *Bcl*I was obtained from Boehringer Mannheim GmbH (Mannheim, Germany). *Hin*fI and *Kpn*I were obtained from Toyobo Co. Ltd. (Tokyo, Japan). The digested DNA fragments were examined by agarose gel electrophoresis as described above.

TABLE 1. Comparison of methods of extracting template DNA

Lane[a]	Strain[b]	Extraction method[c]	Primer pair[d]	Expected PCR product (bp)[d]	Result[e]
A	S-2	1	A	1,108	+
B	S-8	1	B	300	+
C	MPTB	1	C	229	+
D	S-2, 2 colonies[f]	2	B	1,776	−
E	S-2, >10 colonies	2	B	1,776	−
F	S-8	2	B	300	+
G	MPTB	2	C	229	+
H	S-2, 2 colonies	3	A	1,108	+
I	S-2, 2 colonies	3	B	1,776	+
J	MPTB	3	B	300	+
K	S-2, 2 colonies	4[g]	B	1,776	−
L	S-2, 2 colonies	4	B	1,776	−
M	S-2, >10 colonies	4[g]	B	1,776	+
N	S-2, >10 colonies	4	B	1,776	−
O	MPTB, 2 colonies	4[g]	B	300	+
P	MPTB, 2 colonies	4	B	300	+

[a] Lanes in Fig. 1.
[b] S-2, *M. avium* complex, 14141-1395, serovar 2; S-8, *M. avium* complex, 14658-1686, serovar 8; MPTB, *M. avium* subsp. *paratuberculosis*, ATCC 19698.
[c] Method 1, the extraction from the pretreated cells with SDS and the DNA purification were used; method 2, NaOH extraction was used; method 3, the extraction kit for animal or enteric bacterial cells was used; method 4, boiling was done in TE (see Materials and Methods).
[d] The relationship between primer pairs and the expected PCR products is described in Materials and Methods.
[e] +, the expected PCR product existed; −, the expected PCR product did not exist (see Fig. 1).
[f] The starting cell amounts that were extracted.
[g] The boiling time was 8 min instead of 20 min.

RESULTS

DNA extraction method. The template DNAs were extracted from cells of *M. avium* complex serovar 2 and serovar 8 and *M. paratuberculosis* by four DNA extraction methods, and each of them was used for amplification by three sets of primers (Table 1 and Fig. 1). Method 1 supplied high-quality DNA templates suitable for amplifying the large DNA fragments (Fig. 1, lane A). The templates extracted by method 2 and method 4 failed to amplify the large DNA fragments (Fig. 1, lanes D, E, L, and N). Method 3 and the short heating time (8 min) of method 4 with a larger quantity of organisms gave the same results as method 1 (Fig. 1, lanes A to C, H to J, M, and O). Therefore, method 3 was used for further extractions.

Distribution of IS*901* and the novel band in reference strains. By using primers B and template DNAs from 35 reference strains for serotyping, the distribution of IS*901* was

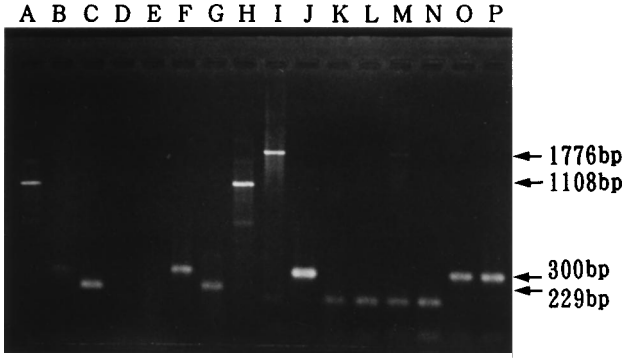

FIG. 1. PCR amplification of MAC DNA under various conditions, as described in Table 1.

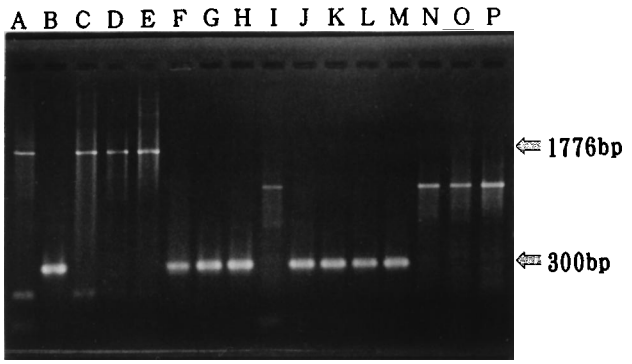

FIG. 2. PCR products amplified by primers B from DNAs of the reference MAC strains extracted by method 3. The 1,776-bp band was derived from the IS*901* sequence, and the 300-bp band was derived from the sequence 150 bp upstream and downstream of the IS*901* insertion site. Lanes: A, ATCC 15769 (serovar 1); B, 11907-300 (serovar 1); C, 14141-1395 (serovar 2); D, 6195 (serovar 3); E, 128-Germany (serovar 3); F, 13528-1079 (serovar 4); G, 4443-1237 (serovar 5); H, 34540 (serovar 6); I, Manten 157 (serovar 7); J, 14658-1686 (serovar 8); K, 6450-204 (serovar 9); L, 1602-1965 (serovar 10); M, 14186-1424 (serovar 11); N, P42 (serovar 12); O, Chance (serovar 13); P, P39 (serovar 14).

examined. The electrophoresis patterns of the PCR products are shown in Fig. 2. Four strains had 1,776-bp bands, indicating the existence of IS*901*, and eight strains had 300-bp bands. The other four strains possessed only one unexpected band that had not been described anywhere previously (Fig. 2, lanes I and N to P).

Four strains of serovars 1 to 3, 10 strains of serovars 1, 4 to 6, 8 to 11, and 21, and 9 strains of serovars 7, 12 to 17, 19, and 20 possessed IS*901*, IS*901* insertion sites, and the novel band, respectively (Table 2). The other strains did not show any other characteristic bands.

Novel band. The size of the novel band was approximately 1,070 bp (Fig. 3, lane 5). The restriction endonuclease fragment patterns of these three types of PCR products (300, 1,070, and 1,776 bp) are shown in Fig. 3. The digestion patterns of the 1,070-bp products by three endonucleases (Fig. 3, lanes 2 to 4) were different from those of 1,776-bp products (Fig. 3, lanes 6 to 8), which corresponded to the patterns that were expected from the reported sequence of IS*901* (6).

Strains from animals and the environment. The distribution of IS*901* in isolates of mycobacteria from swine and the environment was examined by method 3 and with primers B. Primers A were used to confirm the results obtained with primers B. One hundred twenty isolates (Table 3) were examined. No strains had 1,776- or 1,070-bp bands, but 110 strains had the 300-bp band. The pigeon-derived strain possessed IS*901*, but the cow-derived strain did not.

Clinical strains. Fifteen strains of mycobacteria isolated from humans were divided into two strains of *M. avium* with IS*901*, five strains of *M. avium* without IS*901*, two strains of *M. intracellulare* with a 1,070-bp band, and others (Table 4).

DISCUSSION

In order to prepare the template DNA from mycobacteria for PCR, various methods were applied (5, 9, 16). SDS extractions of pretreated cells followed by the phenol and ethanol purifications provided high-quality DNA for the more than 1-kbp products, but these steps were time-consuming (5, 18, 19), which prevented us from easily applying the primers for analysis of the distribution of IS*901*. Heat extraction was easy to perform, but it has been applied only to the amplification of short target DNA sequences (9, 16). We demonstrated that method 3 is a rapid method that can be used with confidence. It gave us the following results.

The details, materials, and mechanisms of method 3 that were originally used for the extraction of DNA from *Esche-*

TABLE 2. PCR products in the laboratory strains

Species and strain[a]	Serovar	Genetic criteria[b]	PCR product (bp) by primers[c]: A	B	C
MAC					
ATCC 15769	1	ND	1,108	1,776	—
11907-300	1	MA	—	300	—
14141-1395	2	MA	1,108	1,776	—
6195	3	MA	1,108	1,776	—
128-Germany	3	MA	1,108	1,776	—
13528-1079	4	MA	—	300	NT
4443-1237	5	MA	—	300	NT
34540	6	MA	—	300	NT
Manten 157	7	MI	—	1,070	—
14658-1686	8	ND	—	300	—
6450-204	9	MA	—	300	NT
1602-1965	10	MA	—	300	NT
14186-1424	11	MA	—	300	NT
P42	12	MI	—	1,070	—
Chance	13	ND	—	1,070	NT
P39	14	MI	—	1,070	NT
Simpson	15	MI	—	1,070	NT
ATCC 15987	16	MI	NT	1,070	NT
Comell	17	ND	NT	1,070	NT
Melnick	18	—	—	—	—
Darden	19	MI	NT	1,070	NT
P40	20	ND	NT	1,070	NT
TMC1419	20	MI	NT	1,070	NT
2993	21	MA	NT	300	NT
51540'Connor	22	—	—	—	—
CDC 1214	23	ND	—	—	—
12645	24	—	NT	680	NT
CDC 1195	25	ND	NT	—	NT
Mackenzie	26	—	NT	—	NT
Lane 3081	27	—	NT	—	NT
Matthews 9055	28	—	NT	—	NT
P-18		ND	1,108	1,776	—
T146	8	ND	—	300	—
M. scrofulaceum					
Bridge	41	—	—	—	—
CDC 1198	42	—	NT	—	NT
Underson	43	ND	NT	—	NT
M. paratuberculosis ATCC 19698		ND	—	300	229
M. bovis BCG		ND	—	—	—
M. fortuitum subsp. *fortuitum* ATCC 6841		ND	—	—	—

[a] The DNA templates for PCR were extracted from these strains by method 3 (see Materials and Methods).

[b] The result of identification according to the genetic criteria described by Saito et al. (11) or Wayne et al. (17). MA, *M. avium*; MI, *M. intracellulare*; —, no reaction; ND, no data.

[c] Primers A specific for *M. avium* RFLP type A/I were derived from the sequence of IS*901*, for which the expected size of PCR products was 1,108 bp. Primers B were derived 150 bp upstream and downstream of the IS*901* insertion site in *M. avium* RFLP type A/I, for which the expected sizes were 300 bp for *M. avium* RFLP type A and *M. paratuberculosis* and 1,776 bp for *M. avium* RFLP type A/I. Primers C specific for *M. paratuberculosis* were derived from the IS*900* sequence, and the expected PCR product was 229 bp. —, no obvious products; NT, not tested.

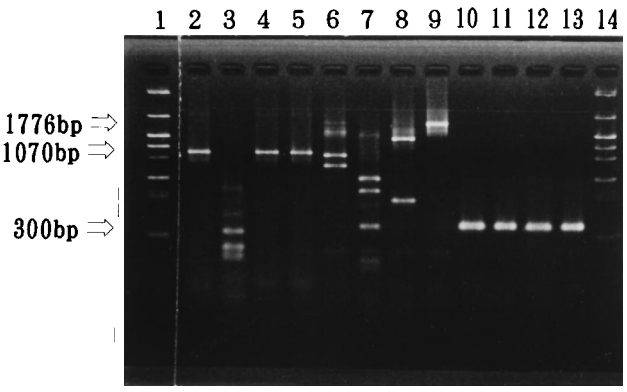

FIG. 3. Restriction endonuclease digestion patterns of PCR products amplified by primers B. Lanes: 1 and 14, pHY Marker, 4,870, 2,016, 1,360, 1107, 926, 658, 489, and 267 bp; 2 to 5, 6 to 9, and 10 to 13, the 1070-bp fragments derived from MAC strain Simpson (serovar 15), the 1776-bp fragments derived from MAC strain 14141-1395 (serovar 2), and 300-bp fragments derived from MAC strain 14658-1686 (serovar 8), respectively; the contents of lanes 2, 6, and 10 were digested with *Bcl*I; those of lanes 3, 7, and 11 were digested with *Hin*fI; and those of lanes 4, 8, and 12 were digested with *Kpn*I.

TABLE 4. Application of primers B to human-derived mycobacterial strains

Species and serovar	No. of strains with the following PCR product by primers B[a]:				
	1,776 bp	1,070 bp	300 bp	—	Total
MAC					
1	1	0	4	0	5
2	1	0	0	0	1
7	0	0	0	1	1
8	0	0	1	0	1
15	0	2	0	0	2
M. scrofulaceum 41	0	0	0	2	2
Untypeable	0	0	3[b]	0	3
Total	2	2	8	3	15

[a] 1,776 bp, possession of IS*901*; 1,070 bp, the novel sequence in *M. intracellulare*; 300 bp, possession of the insertion sites of IS*901* without IS*901*; —, no obvious products.
[b] No serovar-specific glycopeptidolipids.

richia coli or animal cells were not revealed by the manufacturer. Method 2 was used to amplify about 500-bp PCR products (data not shown), and the short extraction time of method 4, which used a large quantity of organisms, was used for the large products with weak signals (Fig. 1 and Table 1). It is speculated that method 3 not only may allow for an easy extraction by the heat destruction of the thick cell walls of mycobacteria but also may provide effective protection against the heat destruction of the extracted DNA (Fig. 1). Even in amplification of small-size DNA (229 and 300 bp), the signals of the amplified DNA on electrophoresis by method 4 were always weaker than those by method 3 (data not shown).

The division of serovars 4 to 20 of MAC into the intermediate group (serovars 4 to 6 and 8 to 11) and *M. intracellulare* (serovars 7 and 12 to 20) was supported by biological and molecular characteristics (1–3, 11). The results obtained with the same strains cited in the report of the study by the International Working Group on Mycobacterial Taxonomy (17) were very similar (Table 2). The strains with 300- or 1,776-bp PCR products amplified by primers B were identified by molecular criteria as *M. avium*, and the strains with the 1,070-bp product were identified as *M. intracellulare*. The other strains including one strain of serovar 18 were not identified by molecular criteria (17). Our results for all strains tested by Saito et al. (11) corresponded to their results (Table 2). In our studies, the reference strains for the serovars of *M. intracellulare* with the novel band did not possess IS*901*, and clinical isolates of *M. intracellulare* possessed 1,070-bp products. Moreover, the novel (1,070-bp) and 1,776-bp products were distinguished by their restriction endonuclease digestion patterns. Therefore, it is likely that primer B might be useful for the identification of *M. avium* and *M. intracellulare* and that *M. intracellulare*-specific sequences may exist in the 1,070-bp products. It is speculated that the sequences around the insertion site may be conserved in both species. The sequences became more interesting in the classification and pathogenesis of MAC.

TABLE 3. Application of primers B to the distribution of IS*901* in swine-derived mycobacterial strains

Species and serovar	No. of strains with the following PCR products by primers B[a]:				
	1,776 bp	1,070 bp	300 bp	—	Total
MAC					
2	0	0	9	0	9
4	0	0	7	0	7
8	0	0	30	1	31
9	0	0	8	0	8
10	0	0	3	0	3
21	0	0	8	1	9
4 and 8	0	0	5	0	5
Others[b]	0	0	9	0	9
Not serotyped	0	0	28	1	29
M. szugai	0	0	0	1	1
Not identified	0	0	3[c]	6	9
Total	0	0	110	10	120

[a] 1,776 bp, possession of IS*901*; 1,070 bp, the novel sequence in *M. intracellulare*; 300 bp, possession of the insertion sites of IS*901* without IS*901*; —, no obvious products.
[b] Other cross-reacting patterns were by serovars 2 and 8, 2 and 9, 3 and 9, 4 and 6, 4 and 9, 6 and 8, and 8 and 9.
[c] These strains had a rough colony morphology.

When plated onto Middlebrook agar, three colony variants of MAC were identified: smooth domed, smooth transparent, and rough colonies (12). The rough colony variant lacked the serovar-specific antigen, resulting in the failure of identification (12). Products of 300 bp were found in the rough variants of reference strains of *M. avium* (data not shown). It seems likely that the three rough strains of not-yet-identified mycobacteria with 300-bp products might belong to *M. avium* (Table 3).

The distributions of the PCR products correlated well with the serovars of isolates except for three veterinary strains of serovars 8, 21, and others and one clinical strain of serovar 7 (Tables 3 and 4). It is presumed that some strains in the third group of MAC (non-*M. avium* and non-*M. intracellulare*) might possess the serovar-specific antigen of *M. avium* (17). Böddinghaus and coworkers (3) showed that serovar 7 is more distant than serovar 18 from serovars 12 to 15, 17, 19 and 20 at the 16S rRNA level, but they used a strain of serovar 7 different from our Manten 157 strain with the 1070-bp product and the same strain as our serovar 18 strain without the 1,070-bp product.

The Manten 157 strain was identified as *M. intracellulare* by DNA-DNA hybridization (2). One of three strains of serovar 7 was not identified as *M. intracellulare* by molecular criteria (17). It may be suggested that the clinical strain of serovar 7 without the 1,070-bp product that we examined belongs to the other cluster of serovar 7 without the 1,070-bp product. Indeed, it was identified as *M. scrofulaceum* by a commercial DNA-DNA hybridization kit (data not shown). Our studies of veterinary and clinical strains validated the ability of primers B to identify *M. avium* and *M. intracellulare*. This method will be established by comparing it with the results of other genetic methods under the same conditions.

All 110 strains of MAC isolated from swine lacked IS*901* and the novel band. We did not obtain strains with IS*901* among the reference strains of the intermediate group, but Kunze and coworkers (5) reported the presence of IS*901* in that group. They also showed that 98 and 94% of strains derived from birds and animals, respectively, possessed IS*901*, but that 33% of 9 swine-derived strains possessed IS*901* (5). Therefore, this result suggests that our results were not in accordance with the results for strains in the intermediate group and the few classical *M. avium* strains and that the lack of possession of IS*901* is characteristic of swine-derived strains such as strains derived from patients with AIDS (5, 7). It is speculated that the factor to which swine were subjected immunocompromised them. This factor, rather than the pathogenic factor, might play a role in the recent swine tuberculosis outbreak, just as AIDS does in tuberculosis in humans.

ACKNOWLEDGMENTS

We thank M. Okabe, Kensei Meat Inspection Center; M. Katsumi, Sendai Meat Inspection Center; A. Sato, Environment and Hygiene Research Center of Kobe City; C. Itakura, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; and T. Tsukahara, Johoku Livestock Hygiene Service Center, Shikamoto, Kumamoto, Japan, for generously providing the veterinary and clinical strains. We also thank W. Baker for critical reading of the manuscript.

REFERENCES

1. **Anz, W., D. Lauterbach, G. Meissner, and I. Willers.** 1970. A comparison of sensitine tests on guinea pigs with serotypes and virulence for chicken in *M. avium* and *M. intracellulare*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **215:**536–549.
2. **Baess, I.** 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **91:**201–203.
3. **Böddinghaus, B., J. Wolters, W. Heikens, and E. C. Böttger.** 1990. Phylogenetic analysis and identification of different serovars of *Mycobacterium intracellulare* at the molecular level. FEMS Microbiol. Lett. **70:**197–204.
4. **Green, E. P., M. L. V. Tizard, M. T. Moss, J. Thompson, D. J. Winterbourne, J. J. McFadden, and J. Hermon-Taylor.** 1989. Sequence and characteristics of IS*900*, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. Nucleic Acids Res. **17:**9063–9073.
5. **Kunze, Z. M., F. Portaels, and J. J. McFadden.** 1992. Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J. Clin. Microbiol. **30:**2366–2372.
6. **Kunze, Z. M., S. Wall, R. Appelberg, M. T. Silva, F. Portaels, and J. J. McFadden.** 1991. IS*901*, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. Mol. Microbiol. **5:**2265–2272.
7. **McFadden, J. J., Z. M. Kunze, F. Portaels, V. Labrousse, and N. Rastogi.** 1992. Epidemiological and genetic markers, virulence factors and intracellular growth of *Mycobacterium avium* in AIDS. Res. Microbiol. **143:**423–429.
8. **Moss, M. T., Z. P. Malik, M. L. V. Tizard, E. P. Green, J. D. Sanderson, and J. Hermon-Taylor.** 1992. IS902, an insertion element of the chronic-enteritis-causing *Mycobacterium avium* subsp. *silvaticum*. J. Gen. Microbiol. **138:**139–145.
9. **Moss, M. T., J. D. Sanderson, M. L. V. Tizard, J. Hermon-Taylor, F. A. K. El-Zaatari, D. C. Markesich, and D. Y. Graham.** 1992. Polymerase chain reaction of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp. *silvaticum* in long term cultures from Crohn's disease and control tissues. Gut **33:**1209–1213.
10. **Nishimori, K., H. Yugi, M. Naiki, T. Sugimura, Y. Tanaka, I. Nonomura, Y. Yokomizo, and S. Kubo.** 1987. Production and characterization of serovar-specific monoclonal antibodies to serovars 4, 8, and 9 of *Mycobacterium intracellulare*. Infect. Immun. **55:**711–715.
11. **Saito, H., H. Tomioka, K. Sato, H. Tasaka, and D. J. Dawson.** 1990. Identification of various serovar strains of *Mycobacterium avium* complex by using DNA probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. J. Clin. Microbiol. **28:**1694–1697.
12. **Schaefer, W. B.** 1980. Serological identification of atypical mycobacteria. Methods Microbiol. **13:**323–344.
13. **Thorel, M. F., M. Krichevsky, and V. V. Levy-Frebault.** 1990. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. Int. J. Syst. Bacteriol. **40:**254–260.
14. **Thoresen, O. F., and F. Saxegaard.** 1993. Comparative use of DNA probes for *Mycobacterium avium* and *Mycobacterium intracellulare* and serotyping for identification and characterization of animal isolates of the *M. avium* complex. Vet. Microbiol. **34:**83–88.
15. **Tsang, A. Y., I. Drupa, M. Goldberg, J. K. McClatchy, and P. J. Brennan.** 1983. Use of serology and thin-layer chromatography for the assembly of an authenticated collection of serovars within the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. Int. J. Syst. Bacteriol. **33:**285–292.
16. **Vary, P. H., P. R. Andersen, E. Green, J. Hermon-Taylor, and J. J. McFadden.** 1990. Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. J. Clin. Microbiol. **28:**933–937.
17. **Wayne, L. G., R. C. Good, A. Tsang, R. Butler, D. Dawson, D. Groothuis, W. Gross, J. Hawkins, J. Kilburn, M. Kubin, K. H. Schröder, V. A. Silcox, C. Smith, M. F. Thorel, C. Woodley, and M. A. Yakrus.** 1993. Serovar determination and molecular taxonomic correlation in *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*: a cooperative study of the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **43:**482–489.
18. **Whipple, D. L., P. Kapke, and C. Vary.** 1990. Identification of restriction fragment length polymorphisms in DNA from *Mycobacterium paratuberculosis*. J. Clin. Microbiol. **28:**2561–2564.
19. **Whipple, D. L., R. B. LeFebvre, R. E. Andrews, Jr., and A. B. Thiermann.** 1987. Isolation and analysis of restriction endonuclease digestive patterns of chromosomal DNA from *Mycobacterium paratuberculosis* and other *Mycobacterium* species. J. Clin. Microbiol. **25:**1511–1515.
20. **Wolinsky, E., and W. B. Schaefer.** 1973. Proposed numbering scheme for mycobacterial serotypes by agglutination. Int. J. Syst. Bacteriol. **23:**182–183.
21. **Yugi, H., H. Nemoto, and K. Watanabe.** 1972. Serotypes of *Mycobacterium intracellulare* of Porcine Origin. Natl. Inst. Anim. Health Q. **12:**168–169.